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Marilyn J. Schneider, Julie A. Montali, Deborah Hazen, and Colby E. Stanton

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ALKALOIDS OF *PICEA*

MARILYN J. SCHNEIDER,* JULIE A. MONTALI, DEBORAH HAZEN, and COLBY E. STANTON

Department of Chemistry, Wellesley College, Wellesley, Massachusetts 02181

ABSTRACT.—Epidihydropinidine [**3**] was isolated from extracts of *Picea engelmannii*. The trans stereochemistry of **3** is of particular interest, as the previously isolated pinidinol [**1**] has cis ring substitution. A survey of *Picea* was conducted to determine the distribution of **3** and **1**. Needles of all species tested contained both alkaloids, except those of *Picea breweriana*, which contained neither. Preliminary tests have indicated a mixture of these alkaloids possesses antifeedant activity against spruce budworm.

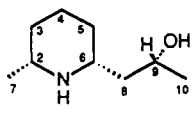
Few members of the family Pinaceae have been reported to contain alkaloids. The alkaloid pinidine has been found in several, but not all species of *Pinus* investigated (1). We have recently reported the first isolation of an alkaloid from *Picea* (spruce). This alkaloid, pinidinol [**1**], was initially identified in the root hemiparasite *Pedicularis bracteosa*. It was then found to have been assimilated from the host, *Picea engelmannii* (Parry) Engelm. (2). We have now identified a second alkaloid from *Pic. engelmannii* and have conducted a survey to determine the distribution of these alkaloids in *Picea*.

RESULTS AND DISCUSSION

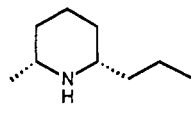
A crude alkaloid extract could be obtained from *Pic. engelmannii*, using either an initial MeOH extraction or steam distillation of plant material. The presence of at least two alkaloids was evident from a ^{1}H-nmr spectrum of the crude alkaloid extract. One major component was identified as pinidinol [**1**] with its characteristic methyl doublets at δ 1.04 and δ 1.16 and H-9 at δ 4.1. Another major component displayed a characteristic methyl triplet at δ 0.92 and methyl doublet at δ 1.07.

Gc-ms of a typical alkaloid extract revealed two major components, one having $[M]^+$ at *m/z* 141 and a longer retention time component with $[M]^+$ at *m/z* 157 (pinidinol [**1**]). Mass spectra of the two components indicated a similar structure, with base peaks at *m/z* 98 and peaks corresponding to $[M-15]^+$. The high volatility of the first component enabled its separation from a crude alkaloid extract by a room temperature bulb-to-bulb distillation in vacuo. ^{1}H-nmr of the purified sample showed only two downfield protons at δ 3.1 and δ 2.9, suggesting the absence of a secondary alcohol as in **1**. The ^{13}C-nmr and ^{13}C DEPT experiments indicated the presence of nine carbons, with two Me, five CH_2, and two carbons attached to nitrogen [δ 50.44 (CH) and δ 45.78 (CH)]. The above spectral data indicated that the basic structure of the *Picea* alkaloid was identical with dihydropinidine [**2**]. The cis stereochemistry was anticipated, given the observed cis arrangement in the related alkaloid pinidinol [**1**] (3).

The hydrochloride salt of the *Picea* alkaloid was prepared, and its properties were compared with literature data and an authentic sample of (±)-dihydropinidine [**2**]·HCl. The *Picea* alkaloid·HCl melted at significantly lower temperature than **2**·HCl: 164.5–165.5°



1



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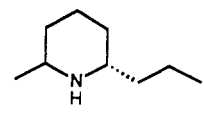

3

vs. 247–248° [(+)-**2**·HCl (1)] or 207–210° [(±)-**2**·HCl (4)]. ^{1}H- and ^{13}C-nmr spectra of the *Picea* alkaloid salt were similar to, but not identical with, literature values (4) and with spectra obtained of a standard sample of (±)-**2**·HCl (Table 1). A ^{1}H-nmr spectrum of a sample containing approximately equal amounts of the *Picea* alkaloid·HCl and (±)-**2**·HCl was the sum of the spectra of the isolated compounds, rather than an average spectrum of the two. Furthermore, gc-ms of a mixture of the *Picea* alkaloid and (±)-**2** revealed two components with different retention times but identical ms. The *Picea* alkaloid is, thus, not identical with dihydropinidine [**2**], and must be the trans isomer, epidihydropinidine [**3**]. Epidihydropinidine [**3**] has been synthesized (5) but has not previously been isolated as a natural product. This finding is of great interest as pinidinol [**1**], previously isolated from *Pic. engelmannii*, was found to have the cis stereochemistry with regard to its piperidine ring substituents (3). The biosynthesis of these alkaloids in *Picea* must then involve an isomerization at some point, possibly via an imine or 9-keto intermediate. The absolute configuration of **3** is under investigation and will be reported elsewhere.

Having established the presence of alkaloids in *Pic. engelmannii*, a next step was to determine whether this species represented an exceptional case or if alkaloids were present throughout the genus. Rushforth has subdivided the genus *Picea* into seven groups (6). At least one *Picea* species was selected from each of these groups and tested for the presence of alkaloids (*Pic. engelmannii* and *Picea pungens* Engelm. represent the same group). Crude alkaloid extracts were prepared of each plant sample using the MeOH extraction method, unless otherwise stated. Each alkaloid extract was analyzed by ^{1}H nmr and gcms for the presence of epidihydropinidine [**3**] and pinidinol [**1**]. It is clear from the results (Table 2) that the presence of alkaloids in *Pic. engelmannii* does not represent an isolated case. With the exception of *Picea breweriana* Watson, epidihydropinidine [**3**] and pinidinol [**1**] were found in all *Picea* tested. *Pic. breweriana*, interestingly, did not appear to contain appreciable amounts of either alkaloid. Rushforth (6) has placed *Pic. breweriana* in a group by itself; the absence of epidihydropinidine and pinidinol is apparently a further indication of the unique character of this species. *Pic. breweriana* may contain other, as yet unidentified, alkaloids; work with this species is continuing.

These results also show that alkaloid presence in *Pic. engelmannii* is not restricted to one plant part. Epidihydropinidine [**3**] and pinidinol [**1**] were

TABLE 1. ^{1}H and ^{13}C nmr Comparison of *Picea* Alkaloid **3**·HCl and (±)-**2**·HCl.

^{1}H nmr (δ)		^{13}C nmr (δ)	
Picea Alkaloid **3**·HCl	(±)-**2**·HCl	*Picea* Alkaloid **3**·HCl	(±)-**2**·HCl
0.95, t, J = 7.2 Hz, 3H, H-10	0.92, t, 3H, H-10	13.65 (Me)	13.64
		16.76 (Me)	18.78
1.3–1.5, m, 2H	1.2–1.6, m, 3H	17.40 (CH_2)	19.42
1.49, d, J = 6.7 Hz, 3H, H-7	1.58, d, 3H, H-7	19.01 (CH_2)	22.95
		26.32 (CH_2)	27.53
1.6–1.8, m, 5H	1.65–2.0, m, 6H	28.86 (CH_2)	30.71
1.9–2.1, m, 3H	2.0–2.2, m, 1H	32.80 (CH_2)	35.18
3.30, br s, 1H, H-6	2.93, m, 1H, H-6	47.95 (CH)	54.53
3.55, br s, 1H, H-2	3.09, m, 1H, H-2	51.53 (CH)	58.43
9.3, br s, 2H, NH	9.1, br s, 1H, NH		
	9.4, br s, 1H, NH		

TABLE 2. Alkaloid Presence in *Picea* and Related Samples.

Sample	Plant part	Compound	
		Epidihydropinidine [3]	Pinidinol [1]
Picea breweriana	needles	−	−
Picea mariana	needles	+	+
Picea chihuahuana	needles	+	+
Picea glauca	needles	+	+
Picea engelmannii	needles	+	+
P. engelmannii	needles (steam distilled)	+	+
P. engelmannii	wood/bark	+	+
P. engelmannii	roots	+	+
Picea pungens	needles	+	+
Arceuthobium microcarpum parasitic on *P. pungens*	foliage	−	+
Picea likiangensis	needles	+	+
Picea brachytyla	needles	+	+

found in samples of needles, wood and bark, and roots.

The hemiparasite *Ped. bracteosa* (2) is not the only plant to take up an alkaloid from *Picea*. *Arceuthobium microcarpum* (Engelm.) Hawksw. and Wiens, a mistletoe parasitic on *Pic. pungens*, was found to contain pinidinol. The absence of epidihydropinidine [3] in the *A. microcarpum* sample may reflect either a selective uptake of alkaloids or evaporative loss of **3** during preparation of this particular sample.

Finally, the presence of alkaloids in *Picea* may have significant biological implications. A variety of alkaloids had been previously tested for antifeedant activity against spruce budworm, even though at the time no alkaloids had been isolated from a spruce budworm host. A range of activity was found among the alkaloids tested (7). Because of the isolation of alkaloids from a spruce budworm host, it seemed appropriate to test **1** and **3** for activity. A preliminary study of a crude alkaloid mixture from *Pic. engelmannii* needles containing pinidinol [1] and epidihydropinidine [3] has indicated a moderate to high antifeedant activity with eastern spruce budworm. (R. Alford, University of Maine, Orono, personal communication). Further work is in progress to study this phenomenon.

EXPERIMENTAL

INSTRUMENTATION.—^{1}H- and ^{13}C-nmr spectra were recorded on an IBM NR/200 nmr spectrometer. ^{13}C DEPT spectra were recorded on a Bruker AC-P 300 nmr spectrometer. Optical rotations were obtained with a Rudolph Autopol II automatic polarimeter. Mp's were determined with a Meltemp capillary melting point apparatus and are uncorrected. Gc-ms was performed using a Hewlett-Packard model 5995 gc-ms with a 59970B workstation. Gc column: 12 m × 0.20 mm × 0.33 μm film HP-1 crosslinked methyl silicon fused silica. Temperature program: 50° (2 min), heating rate 10°/min to 200°. He carrier gas was used with splitless injection.

PLANT MATERIAL.—Plant collections are listed in Table 3. All plant material was from the Arnold Arboretum of Harvard University except for the following: *Pic. engelmannii* wood/bark and a foliage sample, as well as *Pic. pungens*, were identified by D.H. Wilken (Dept. of Biology, Colorado State University). *A. microcarpum* was identified by F. Hawksworth (USDA Forest Service, Fort Collins, CO). All collected plant material was stored at 4° until needed.

STEAM DISTILLATION AND ALKALOID ISOLATION FROM *PIC. ENGELMANNII* NEEDLES.—The general procedure of Juneau (8) was followed. *Pic. engelmannii* needles (50.9 g) were ground in a Waring blender with 8% Na_2CO_3 (150 ml). After a further addition of Na_2CO_3 solution (125 ml) and H_2O (250 ml), the mixture was steam-distilled. The distillate (ca. 250 ml) was made basic by the addition of 50% KOH and extracted with $CHCl_3$ (7 × 25 ml). The $CHCl_3$ phase was extracted with 2N HCl (6 × 25 ml). Basification of the aqueous phase with 50% KOH, extraction

TABLE 3. Plant Collections.

Plant	Population	Voucher No.
Pica breweriana	Arnold Arboretum	1218-79A
Picea mariana	Arnold Arboretum	1336-78A
Picea chihuahuana	Arnold Arboretum	734-78A
Picea glauca	Arnold Arboretum	1145-72D
Picea engelmannii		
needles	Arnold Arboretum	16477-D
needles, wood/bark	Cameron Pass, CO	CSU 8789
roots	Arnold Arboretum	1310-79-F
Picea pungens	Fort Collins, CO	FRS 416
Arceuthobium microcarpum	Springerville, AZ	J. Sprackling SN
Picea likiangensis	Arnold Arboretum	461-80-E
Picea brachytyla	Arnold Arboretum	1409-82

with $CHCl_3$ (7 × 20 ml), and evaporation of the $CHCl_3$ gave a crude alkaloid extract (76 mg). This sample was analyzed by ^1H nmr; the spectrum contained a triplet at δ 0.92 and a doublet at δ 1.07 (epidihydropinidine [**3**]), as well as doublets at δ 1.04 and δ 1.16 (pinidinol [**1**]). Gc-ms indicated the presence of two alkaloids. Component 1 (epidihydropinidine [**3**]): Rt 6.9 min; *m/z* (rel. abundance) $[M]^+$ 141 (1), 140 (1), 126 (6), 99 (6), 98 (100), 70 (9). Component 2 (pinidinol [**1**]): Rt 9.5 min; *m/z* (rel. abundance) $[M]^+$ 157 (3), 142 (10), 98 (99), 82 (8), 70 (15).

ISOLATION OF EPIDIHYDROPINIDINE [**3**] FROM *PIC. ENGELMANNII* NEEDLES.—A crude alkaloid extract (119 mg) was obtained from *Pic. engelmannii* needles (55 g) following the above procedure. Epidihydropinidine (57 mg) was distilled from the alkaloid extract at room temperature in vacuo (24.5°, 2 mm). ^1H nmr ($CDCl_3$) δ 3.1 (qdd, $J=6.6$, 6, 3 Hz, 1H, H-2), 2.9 (m, 1H, H-6), 1.7–1.4 (m, 5H), 1.4–1.2 (m, 6H), 1.08 (d, $J=6.6$ Hz, 3H, H-7), 0.92 (t, $J=7$ Hz, 3H, H-10); ^{13}C nmr ($CDCl_3$) δ 50.44 (CH), 45.78 (CH), 36.31 (CH_2), 32.99 (CH_2), 30.80 (CH_2), 21.10 (Me), 19.53 (CH_2), 19.46 (CH_2), 14.02 (Me). The hydrochloride was prepared by treating an Et_2O solution of the alkaloid with gaseous HCl, evaporating the solvent, and recrystallizing with EtOAc to give 19 mg, mp 164.5–165.5° [lit. (5) 134–135°, Et_2O/MeOH]; $[\alpha]^{29}D$ +4.7 ($c=3.8$, EtOH); ^1H and ^{13}C nmr see Table 1.

MeOH EXTRACTION AND ALKALOID ISOLATION FROM *PICEA*.—Needles of *Picea glauca* (Moenchen) Voss (22.5 g) were ground in a mortar and pestle under liquid N_2. The ground residue was extracted with MeOH (3 × 250 ml) over a total period of 12 days. The filtered and evaporated MeOH extract was taken up in H_2O (125 ml) and extracted with Et_2O (6 × 25 ml) and with $CHCl_3$ (5 × 25 ml). The aqueous phase was basified with K_2CO_3 and extracted with $CHCl_3$ (5 × 25 ml). Evaporation of the $CHCl_3$ gave a crude alkaloid extract (58 mg). This sample was analyzed by ^1H nmr; the spectrum contained a triplet at δ 0.92 and a doublet at δ 1.07 (epidihydropinidine), as well as doublets at δ 1.04 and δ 1.16 (pinidinol). Gc-ms indicated the presence of epidihydropinidine [**3**] and pinidinol [**1**] as the two major alkaloids.

Following the above procedure, the remaining plant samples (Tables 2 and 3) were extracted. In some cases, an additional acid/base extraction step was used to remove remaining aromatic contaminants. All samples were needles, unless otherwise specified; the following results were obtained: Sample (mass plant material extracted, mass crude alkaloids obtained); *Pic. breweriana* (41.7 g, 37 mg), *Picea mariana* (Miller) B.S.P. (18.0 g, 20 mg), *Picea chihuahuana* Martinez (20.0 g, 60 mg), *Pic. engelmannii* (100 g, 33 mg), *Picea engelmannii* wood/bark (32 g, 5 mg), *Pic. engelmannii* roots (32.4 g, 43 mg), *Pic. pungens* (10 g, 14 mg), *A. microcarpum* foliage (15.0 g, 33 mg), *Picea likiangensis* (Franch.) Pritzel (21.0 g, 14 mg), *Picea brachytyla* (Franch.) Pritzel (20.0 g, 6 mg).

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